

OVARY TRANSPLANTS IN DROSOPHILA MELANO- GASTER: STUDIES OF THE CHARACTERS SINGED, FUSED, AND FEMALE-STERILE

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INTRODUCTION

In *Drosophila melanogaster* several mutant types are known which are characterized by female sterility. Three of these, singed (*sn*, 1-21.0; bristles and hairs have singed appearance), fused (*fu*, 1-59.5; longitudinal veins 3 and 4 fused at base), and female-sterile (*fes*, 2-6 ±; ovaries undeveloped), have been studied by transplanting ovaries from mutant flies to wild type hosts and vice versa in order to determine whether, under these conditions, there is any detectable influence of the tissues of the host on the development of the implant.

In connection with the transplants involving singed, an attempt was made to determine the influence of certain variations in genetic constitution and of relative development on the frequency with which an implanted ovary becomes attached to the genital ducts of the host. These studies of frequency of attachment were made primarily for the purpose of providing a measure of the usefulness of the method of gonad transplantation in *Drosophila* as an experimental tool.

MATERIALS AND METHODS

The methods of collecting eggs, culturing larvæ, and of making the transplantation operations were essentially similar to those given by Ephrussi and Beadle (1936). Unless stated otherwise, all transplantation operations were made on individuals in the late larval stage of development within 24 hours of puparium formation, and donor and recipient larvæ were of approximately the same absolute age.

Stocks of the three mutant types were made up in such a way that an eye color gene, known to affect pigmentation of the Malpighian tubes (Beadle, unpublished), was carried in the same chromosome as that in which the sterility gene in question is located. In this way larvæ homozygous for the sterility gene could be selected at the time the operations were made. The actual stocks used were $\tau^w sn/CIB$ \times $\tau^w sn$, $\tau^w fu/CIB$ \times $\tau^w fu$, and *fes* *lt*/*Cy*, *sp*² \times *fes* *lt*.

In those experiments in which it was desired to determine the nature of the eggs laid by a given female, mature females into which ovaries had been implanted were placed, singly, in vials, given fresh food, mated to males of the appropriate genotype, and observed for three or four days in order to note the kind of eggs laid and to collect any larvæ that hatched from them. Larvæ from individual females were transferred to culture bottles for determination of phenotype. At the end of three or four days each female was dissected and a record taken of the number of ovaries and the way in which they were attached to the oviducts.

In the experiments dealing with frequencies of attachment of implanted ovaries, advantage was taken of the fact that singed ovaries can be distinguished from wild type ovaries by the characteristic shape of mature or nearly mature eggs (shorter and blunter at the anterior end than are eggs produced in a wild type ovary). Thus, by aging mature females for two or three days, the eggs in the ovaries develop sufficiently so that, by dissection, it is a simple matter to determine whether or not the implanted ovary is attached to the oviduct of the host.

TRANSPLANTATIONS INVOLVING SINGED

Females homozygous for the gene *singed* are known to produce eggs visibly different in shape from those produced by wild type females; such eggs never hatch (Mohr, 1922). Singed males show normal fertility, and oöcytes of the constitution $sn/+$ give rise to reduced eggs, sn in constitution, which develop normally after fertilization with either sn^+ or sn sperm. It is clear, then, that the two types of eggs, sn produced by a sn female and sn produced by a $sn/+$ female, are differentiated by the constitution of the mother. This differentiation might be brought about directly in the developing oöcyte or indirectly by means of an influence of other maternal tissues. The experiments reported below were carried out in an attempt to differentiate between these two possibilities.

Ovaries from $\tau^0 sn$ larvæ were transplanted to F_1 wild type larvæ derived from the cross of the two inbred wild stocks, Florida and Swedish-c. From 37 larvæ into which had been injected $\tau^0 sn$ ovaries, 27 gave rise to adult females. These were mated individually to $\tau^0 sn$ males. Two females were lost before any record of the eggs was made, and one before dissection. Of the 24 females on which complete records were secured, it was found that 13 laid two types of eggs, one type judged by appearance to be sn , the other wild type. The remaining 11 females laid only wild type eggs. When these females were

dissected, it was found that the attachment of the ovaries to the ducts agreed in every case with the egg-laying record (Table I). Of the 11 females which laid only wild type eggs, 5 had no developed implanted ovary. Only eggs of normal wild type appearance hatched and all hatched eggs that were tested by growing the larvæ to maturity gave rise to wild type flies. It is clear that none of these normal eggs originated in the implanted *sn* ovary.

TABLE I

Summary of the results of transplanting singed ovaries to wild type female hosts and the reciprocal. In this and following table wild type is designated by +.

Implant	Host	Number of mature females	Eggs laid	Total progeny		Result of dissection, implant		
				Phenotype	Number	Attached	Free	Not determined
<i>w^esn</i>	+	13	+ and <i>sn</i>	+	746	13*	0	
<i>w^esn</i>	+	6	+	+	624	1†	5	
+	<i>w^esn</i>	6	+ and <i>sn</i>	+	321	4	0	2
+	<i>w^esn</i>	4	+	+	26	2	0	2
+	<i>w^esn</i>	3	<i>sn</i>	—	0	0	2	1

* In one female three ovaries were attached to ducts, in a second, only one wild type ovary present; see footnote to Table II.

† Three ovaries attached to ducts; see footnote to Table II.

In making the reciprocal transplants, wild type ovaries transplanted to *w^esn* larvæ, 34 operations were made. Seventeen adult females were obtained which were mated to *w^esn* males. A record of the eggs laid was secured on 13, and the attachment of the ovaries on 8. The *w^esn* females were less vigorous than wild type females and were inclined to stick to the food mass or on the moist walls of the vials. As indicated in Table I, the egg-laying record and the dissection records agree in all cases. Here again only the eggs which were judged to be wild type gave rise to offspring and these were all wild type; they must have originated in wild type implanted ovaries. This result shows that, aside from the production of abnormal eggs, the reproductive apparatus of a singed female is able to function in an essentially normal way, i.e., the genital ducts, accessory glands, and external genitalia of such a female are functionally normal.

It is evident from the results just described that there was no detected influence of host on implant or of implant on host, i.e., the implants developed according to their own genetic constitutions with no apparent influence of the genetic constitution of the host tissues. So far as the experiments go, the differentiation is intragonadal in nature,

but the possibility of extra-gonadal influences, acting before the time at which the transplants were made, is not excluded.

TRANSPLANTATION OF FUSED OVARIES

Fused females lay eggs of normal appearance but which fail to hatch when fertilized by sperm from fused males. According to Lynch (1919) such eggs show signs of development and in very exceptional cases (2 in several hundred) may give rise to larvæ which die at an early stage of development. Lynch (l. c.) observed, however, that if such eggs are fertilized by X sperm from a wild type male (or other not-fused males) normal development occurs. Absence of males from such matings indicates that fertilization by a Y sperm does not bring about normal development. Eggs from $fu/+$ females, even though they be fu in constitution after reduction, show normal development when fertilized with normal X, fused X, or Y sperm. Fused males are apparently normal in fertility. As pointed out by Lynch, a fu egg can be made good by something which happens to it before fertilization (development from a $+/fu$ oöcyte) or, if it develops from a fu/fu oöcyte, by being fertilized by a not-fused X sperm. Assuming that the effect of the fu^+ gene is a positive one, we might express this in terms of gene activity in the following way. A fu egg arising from a fu/fu oöcyte lacks something essential for its normal development. If a fu egg arises from a $fu/+$ oöcyte, this deficiency has been made good before fertilization by the activity of the fu^+ gene directly in the oöcyte (possibly after fertilization by the activity of the fu^+ gene in the polar body nuclei), or indirectly by the activity of the fu^+ gene in cells other than the oöcyte. Whatever is deficient in a fu egg arising from a fu/fu oöcyte can evidently be compensated for by the activity of the fu^+ gene brought in by an X sperm. The results of transplanting a fu ovary to a not-fused female should answer the question of whether the fu^+ gene can influence the oöcytes indirectly through tissues outside the ovary.

White fused ($w\ fu$) ovaries were implanted in $w^c sn$ hosts. Singed females were used as hosts so as to be able to distinguish the eggs of an implanted ovary from those of the ovaries of the host. Of 37 $w^c sn$ females in which fused ovaries had been implanted, 18 laid both singed and fused eggs, 3 fused eggs only, 14 singed eggs only, and in 5 cases no eggs at all were laid. Females laying fused eggs and mated to fused males gave no offspring. After three days, 6 females that had been laying fused eggs were isolated and remated to wild type males. From the eggs laid during the next three days some larvæ hatched.

These were collected and placed in a culture bottle en masse. In all, 28 flies emerged from larvæ collected in this manner; all were wild type females.

In this experiment, as in the case of the analogous experiment with singed ovaries, the implanted fused ovaries behaved in all respects in the same way as would have been expected had they completed development in their original environment. Conclusions similar to those arrived at in the experiments with singed are indicated.

EXPERIMENTS WITH FEMALE-STERILE

Females homozygous for the gene *fes* have rudimentary ovaries; they can be distinguished from normal females by dissection one day or more after eclosion. Apparently the oöcytes fail to grow normally. Homozygous *fes* males are fertile.

Transplantation of wild type ovaries to *fes* females show that such an ovary can become attached to an oviduct of the host and function normally. Of 7 such females in which the implanted ovary had developed, 2 produced wild type eggs which, when fertilized with sperm from *fes* males gave rise to wild type adult flies. Five females showed the implanted ovary normally developed but unattached.

Of 4 wild type females in which implanted female-sterile ovaries had been implanted, 2 had the implant attached and in 2 the implant was unattached. In both cases, after aging of the females, the implanted ovaries showed no more development than is characteristic for the ovaries of normal *fes* females. It is clear from the two instances in which an implanted *fes* ovary replaced a normal wild type ovary of the host that *fes* ovaries are capable of competing successfully in attachment with normal ovaries.

The experiments with female sterile ovaries show that, under the conditions of the experiments and with respect to the characters under consideration, the development of ovary implants is autonomous or independent of the genetic constitution of the host.

In experiments in which it is desired to recover eggs from ovaries grown in hosts of a different genetic constitution, the character female-sterile promises to be of considerable value. When one uses *fes* females as hosts, no eggs develop in the ovaries of the host and all recovered eggs therefore originate in the implanted ovary. Furthermore, the limited development of *fes* ovaries minimizes the unfavorable effects of mechanical crowding often apparent in females with three normally developed ovaries. Females homozygous for the *fes* gene with an implanted normal ovary have been observed to lay an average of more

than 25 eggs per day for an interval of 10 days with no evident signs of decreased production at that time.

FREQUENCY OF ATTACHMENT OF IMPLANTED OVARIES

As pointed out above it is possible, by using donors and recipients which carry a gene difference at the singed locus, to determine by dissection of aged females whether or not an implanted ovary has established connection with an oviduct of the host.

Experiments were carried out which have a bearing on three questions: (1) Is a singed ovary on a par with a wild type ovary in establishing a connection with an oviduct? (2) Do variations in the difference in genetic constitution between ovaries competing for attachment influence the result? (3) What is the effect, on competition for attachment, of a difference in development of competing ovaries (age difference between donor and recipient)? Three stocks were used:

1. F_1 $\tau^c sn$ females from the cross of two distantly related $\tau^c sn$ stocks, $\tau^c sn / CIB$ females mated to $\tau^c sn$ males from the stock $\bar{y}^c \times \tau^c sn$.
2. F_1 wild type females from the cross of Florida and Swedish-c inbred wild type stocks.
3. Inbred wild type stock Oregon-R-c, made homozygous an unknown number of generations previously by the standard inversion technique.

The results of two sets of reciprocal transplants involving these stocks are summarized in Table II. In the first pair of reciprocal transplants, involving the two outcrossed stocks, the percentages of attachment are 75.0 and 65.3. Neither of these is significantly different statistically from the 66.7 per cent expected on the basis of random attachment, and both are higher than the percentage (45.4) found by Ephrussi and Beadle (1935) in a series of miscellaneous experiments. Furthermore, the difference between the two values is not statistically significant. The second pair of reciprocal transplants, involving the outcrossed $\tau^c sn$ stock and the inbred Oregon-R-c wild type stock give percentages of attachment of implanted ovaries of 44.7 and 41.7. These two values, again approximately equal, are significantly lower than the corresponding values obtained in the first pair of reciprocals, and are significantly lower than the 66.7 per cent expected on the assumption that attachment is random.

It can be seen that in each pair of reciprocal transplants the frequency of attachment of wild type implants is higher than that of singed implants. However, in each instance the difference is of doubtful statistical significance. Combining the two series so as to compare wild

type in *w^esn* with *w^esn* in wild type, values of 65.3 and 53.6 per cent are obtained, a difference approximately 2.5 times its probable error. While it cannot be concluded that there is no inherent difference between singed and wild type ovaries with respect to the chance of their becoming attached to oviducts of the host, the experiments fail to demonstrate such a difference and show that, if it does exist, it must be of relatively little importance as compared with other differences.

From the data tabulated in Table II it is evident that the results are different with the outcrossed and inbred wild type stocks. Furthermore, reciprocal transplants give approximately equal frequencies of

TABLE II

Summary of data from experiments on frequency of attachment of implanted ovaries. In all instances listed at least two ovaries were attached to the two lateral oviducts of the host. In the calculated frequencies of attachment only the two classes with three ovaries developed, two attached, and one free, were taken into account. Probable errors are given with the calculated percentages.

Implant	Host	Three ovaries			Two ovaries *		Percentage attachment of implant
		Implant free	Implant attached	All attached *	Implant attached	No implant	
+Fla/+S-c	<i>w^esn</i>	15	45	5	2	12	75.0±3.8
<i>w^esn</i>	+Fla/+S-c	17	32	6	1	13	65.3±4.6
+Ore-R-c	<i>w^esn</i>	26	21	4	2	2	44.7±4.9
<i>w^esn</i>	+Ore-R-c	28	20	1	0	7	41.7±4.8

* In this and Table III, 26 instances are recorded of attachment of three ovaries. Two additional cases of such attachment are recorded in the footnotes to Table I. In many of the attachments of this nature one ovary appeared to be imperfectly attached. In no instance was a clear bifurcation of a lateral oviduct observed; often two ovaries appeared to have a common attachment to a single lateral oviduct. In several recorded instances only two ovaries were developed, sometimes both from the host (failure of the transplantation operation or possibly failure of the implanted ovary to develop), sometimes only one from the host (presumably injury to or destruction of a normal ovary during operation).

attachment of the implant, high in one case and low in the other. It is pointed out again that in these experiments the donors and recipients were approximately equal in absolute age (ages controlled to within a period of 2 hours or less).

It seems reasonably safe to assume that the differences in frequency of implant-attachment observed between the two wild type stocks is to be attributed to differences in genetic constitution. The difference between the two series and the approximate equality in attachment frequency in reciprocal transplants suggest, and are consistent with, the following assumptions:

1. Which two of three ovaries will become attached is a matter of chance if the ovaries of the host and the implanted ovary are at the same developmental stage at the time of attachment.

2. If the implanted ovary is at a different developmental stage from the ovaries of the host (either more or less advanced) at the time of attachment, the implant will be at a disadvantage in competition with the ovaries of the host.

3. Development of ovaries takes place at different rates relative to absolute age in females of different genetic constitutions.

It should be possible to test directly the first two of these assumptions by varying the relative ages of donors and recipients. There is no way of determining except by trial whether the low value obtained in the series involving the Oregon-R-c wild type stock is the result of

TABLE III

Results of transplants involving *w^esn* and Oregon-R-c (Ore-R-c) with an age difference between donor and recipient.

Implant	Host	Implant younger or older	Age difference	Three ovaries			Two ovaries		Percentage attachment of implant
				Im-plant free	Im-plant at-tached	All at-tached	Im-plant at-tached	No im-plant	
Ore-R-c . . .	<i>w^esn</i>	Younger	<i>hours</i> 16-23	6	27	2	2	11	81.8±4.5
<i>w^esn</i>	Ore-R-c	Older	16-24	0	1	1	0	0	—*
<i>w^esn</i>	Ore-R-c	Younger	21-26	53	53	7	0	16	25.4±3.5

* Hosts died after pupation, see text.

slower or of faster development of the ovaries of Oregon-R-c relative to those of the *w^esn* stock. If the former is the explanation, then by implanting ovaries from Oregon-R-c females into older *w^esn* females, the frequency should be decreased. If the latter is the case, then ovaries from Oregon-R-c females implanted into older *w^esn* females should show an increased frequency of attachment. Four combinations with a given age-difference are obviously possible with two given stocks.

Table III summarizes the attachment frequencies of implants in experiments made with the Oregon-R-c and *w^esn* stocks where the average age difference between donor and recipient was approximately 20 hours. It is seen that Oregon-R-c ovaries transplanted to older *w^esn* females give a significantly higher frequency of attachment than was obtained in the comparable experiment without an age difference (81.8 per cent as compared with 44.7 per cent). The reciprocal of this trans-

plant, $\omega^e sn$ ovaries in younger Oregon-R-c females, should, following the assumptions made above, likewise give a high attachment frequency. However, when the experiment was made it was found that the hosts lived for more than 24 hours, appeared to pupate normally, but for the most part died before maturity. Four sets of transplants were made at separate times, and in a total of 149 operations, only two females reached maturity. As compared with this high mortality, the average mortality for all other experiments involving an age-difference was 32.2 per cent; the mortality for the transplantation operations involving no age-difference was 45.5 per cent. Apparently, for some reason, the implant was lethal to the host in this particular case.

In the only other combination attempted, $\omega^e sn$ ovaries implanted in older Oregon-R-c hosts, the frequency of attachment was lower than in the same combination without an age difference (25.4 as compared with 41.7 per cent, a difference of approximately 2.9 times its probable error).

The two successful combinations with an age difference are consistent with the assumptions listed above and indicate that, if these assumptions are correct, the ovaries of Oregon-R-c females are developmentally further advanced than are those of $\omega^e sn$ females of a corresponding absolute age. However, in the absence of the reciprocals of these age-difference experiments, the assumptions are by no means proved to be correct. The lethal result in the one combination is quite unexplained and suggests either that the assumptions are incorrect or that they fail to take into account all of the factors concerned.

SUMMARY

The development of singed ovaries transplanted to wild type females in the late larval stage shows autonomous development. Eggs recovered from such females have the characteristic shape of eggs from singed females and they fail to give rise to larvæ. Wild type ovaries grown in singed hosts likewise show autonomous development. Viable eggs can be recovered from such ovaries; they give rise to wild type offspring (females heterozygous for $\omega^e sn$) when fertilized by $\omega^e sn$ sperm.

Fused ovaries grown in singed hosts have characteristics not detectably different from such ovaries grown in their normal position. Recovered eggs fertilized by *fu* or by Y sperm fail to hatch, but those fertilized by not-*fu* X-carrying sperm give rise to normal females heterozygous for *fu*.

Ovaries from female-sterile females grown in wild type hosts may become attached to the oviducts of the hosts, competing successfully

with normal ovaries, but they remain rudimentary as they do in their normal genetic surrounding. Wild type ovaries grown in female-sterile females show autonomous development. Viable eggs giving normal development are recovered following attachment of the implant to the oviduct of the host.

Using a single outcrossed stock of $\alpha^{c}sn$ and two wild type stocks, one outcrossed and one inbred, reciprocal ovary transplants show: (1) that the frequency of attachment of the implant varies with different genetic stocks, and (2) that, under the conditions of the experiments and with the numbers involved, there is no statistically significant difference in the frequency of implant-attachment in reciprocal transplants.

Transplants in which donors and recipients were different in absolute age show that the frequency of attachment of the implant can be varied, either increased (in certain combinations) or lowered, by varying the relative ages of donors and recipients. In one combination in which ovaries were implanted to hosts younger than the donors, there was apparently a lethal interaction such that most of the hosts died after pupation. The bearing of the age-difference experiments on the differences in frequency of implant-attachment observed with different stocks is considered.

The application of certain of the results summarized above to the use of gonad transplants in *Drosophila* as an experimental tool are pointed out.

LITERATURE CITED

- EPHRUSSI, B., AND G. W. BEADLE, 1935. La transplantation des ovaires chez la *Drosophile*. *Bull. Biol. Fr. Belg.*, **69**: 492.
- EPHRUSSI, B., AND G. W. BEADLE, 1936. A technique of transplantation for *Drosophila*. *Am. Nat.*, **70**: 218.
- LYNCH, C. J., 1919. An analysis of certain cases of intra-specific sterility. *Genetics*, **4**: 501.
- MOHR, O. L., 1922. Cases of mimic mutations and secondary mutations in the X-chromosome of *Drosophila melanogaster*. *Zeitschr. ind. Abst. u. Vererb.*, **28**: 1.